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Mist, substrate water potential and cutting water potential influence rooting of stem cuttings of loblolly pine

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Summary We investigated the influence of cutting water potential (Ψ_{cut}) on rooting of juvenile hardwood (dormant) and softwood (succulent) stem cuttings of loblolly pine (*Pinus taeda* L.) propagated under varying substrate water potentials (Ψ_{sub}) and volumes of mist application. Mist treatment and Ψ_{sub} contributed to the Ψ_{cut} of unrooted stem cuttings. When Ψ_{sub} was held constant across mist treatments, mist treatment contributed strongly to Ψ_{cut}. Substrate water potential affected rooting percentage when mist treatment was sub-optimal or excessive, otherwise mist treatment had a stronger effect than Ψ_{sub} on rooting percentage. Cuttings rooted best when subjected to moderate cutting water potentials (–0.5 to –1.2 MPa) during the initial 4 or 5 weeks of the rooting period. Cuttings experiencing either severe water deficit or no water deficit rooted poorly. We conclude that the rooting environment should impose a moderate water stress on loblolly pine stem cuttings to achieve optimum rooting.

Keywords: adventitious rooting, clonal forestry, Pinus taeda, vegetative propagation, soil water deficit, water relations, water stress.

Introduction

Propagation of loblolly pine (*Pinus taeda* L.) by juvenile stem cuttings, produced on recurrently sheared stock plants (hedges), can be used to multiply superior full-sib families and elite clones within superior families for reforestation (Zobel and Talbert 1984, Goldfarb et al. 1997, Frampton et al. 1999, 2000). Production of high-quality rooted stem cuttings on a large scale, however, requires maintenance of a suitable rooting environment over large areas. Rooting environments can vary from polyethylene-covered greenhouses to semi-shaded structures and direct field-setting of stem cuttings. Thus, specific irrigation protocols may not be applicable in all rooting environments (Frampton and Hodges 1989, Rowe et al. 1999, Gocke et al. 2001). Therefore, a more physiological approach to understanding water relations of stem cuttings during the rooting period may aid in the design and control of suitable propagation environments.

In most commercial systems for rooting of stem cuttings, intermittent mist application minimizes plant water stress by lowering leaf temperature and transpiration, thereby increasing the humidity surrounding the cuttings (Tukey 1978). It has been shown that the volume of water applied during intermittent mist application can affect rooting percentage (Greenwood et al. 1980, Loach 1988). The water content of the substrate (rooting medium) is also important during rooting of woody ornamental species (Rein et al. 1991), especially when mist is not applied (Graves and Zhang 1996) or applied infrequently to softwood cuttings (Giroux et al. 1999).

The water potential of a stem cutting (Ψ_{cut}) is a physiological indicator of the water deficit (Kramer and Kozlowski 1979) that results when transpiration rate exceeds water uptake (Grange and Loach 1983, Bray 1997). Although available water is not usually limiting during rooting, high negative Ψ_{cut} values may arise, presumably because of the absence of a root system for water uptake. For Ψ_{cut} to increase, stem cuttings must either absorb applied mist through foliage or absorb water through the basal portion of the cutting, which is inserted into the substrate. Although both modes of uptake have been reported to occur in stem cuttings of radiata pine (*Pinus radiata* D. Don.) (Cameron and Rook 1974, Cremer and Svensson 1979), the modes of uptake that contribute to Ψ_{cut} of loblolly pine under different environmental conditions have not been reported.

It has been suggested that rooting percentage is related linearly to Ψ_{cut} during the rooting period (Loach 1977, Loach and Whalley 1978, Hartmann et al. 2002). This relationship implies that stem cuttings with a high Ψ_{cut}, will have an increased likelihood of root initiation and development. On the other hand, supra-optimal mist application, which decreases water stress, can decrease rooting (Greenwood et al. 1980, Harrison-Murray and Howard 1998). Decreased rooting under conditions of minimal water stress has also been observed and is usually attributed to reduced oxygen concentrations near root initials (Loach 1985, Soffer and Burger 1988) or, secondarily, to a buildup of disease-causing fungi (Hartmann et al. 2002). Alternately, Harrison-Murray and Howard (1998) suggested that some degree of water stress is required for adventitious root formation to occur. Murthy and Goldfarb (2001) observed that moderate to severe short-term water stress in stem cuttings of loblolly pine, before transfer to optimal conditions, decreased percent rooting only slightly. The degree of water stress that stem cuttings can experience without serious inhibition of rooting percentage is poorly defined.

In previous studies, the effects of gradients in mist and substrate water content on rooting percentage were investigated by positioning stem cuttings at various distances from a fixed mist nozzle placed above the rooting bed (Harrison-Murray and Howard 1998, Loach 1988). Although indicative of the effect of the overall amount of water applied, this experimental design precludes differentiation between the effects of the mist treatment and those of substrate water content, because the factors are confounded. However, a more detailed understanding of the effects of aerial mist and substrate water content and their interactions on Ψ_{cut} and rooting would facilitate implementation of irrigation systems that would be applicable across rooting environments.

We studied the relationship between rooting percentage and Ψ_{cut} in juvenile hardwood (dormant) and softwood (succulent) loblolly pine stem cuttings and determined how this relationship is affected by aerial mist and substrate water potential (Ψ_{sub}). Specifically, we examined the effects of the volume of mist delivered per application (mist treatment) and Ψ_{sub} on Ψ_{cut} and root formation. We also tested the effects of various mist treatments on Ψ_{cut} and root formation, with Ψ_{sub} held constant. In the analysis of all experiments, Ψ_{cut} was treated as an independent variable to determine its relationship with rooting percentage.

Materials and methods

Plant material

In June 1996, 200 seeds from two unrelated full-sib families (100 seeds per family) of a Florida provenance of loblolly pine were germinated. In May 1997, individual seedlings were transplanted to 12-l containers filled with a substrate of composted, shredded pine bark:sand (4:1 v/v). All stock plants were grown outdoors at the North Carolina State University Horticultural Field Laboratory (35°47′ N, 78°39′ W). At the beginning of March and August of each year, seedlings were sheared to a height of about 15 cm and maintained as hedged stock plants (hedges) by removing all remaining terminal buds of lateral stems in accordance with protocols of Cooney and Goldfarb (1999). Irrigation was provided, as needed, by an overhead sprinkler system. About 51 g of commercial slow-release fertilizer (18:6:12 N,P,K; Osmocote, 8–9-month, Grace-Sierra, Milpitas, CA) was applied twice per year to each hedge after pruning to maintain tissue nitrogen concentrations at the target values recommended by Rowe et al. (2002). Other macro- and micronutrients were applied as indicated by periodic foliage analyses following recommendations for loblolly pine seedlings (C.B. Davey and J.B. Jett, Dept. of Forestry, North Carolina State University, Raleigh, NC, personal communication). Hand weeding was performed as needed and several pesticides were applied throughout the growing season to control tip moth (*Rhyacionia frustrana* Comst.) and fusiform rust (*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*).

In February 1999, ramets were produced from the original seedling ortets by rooting stem cuttings from each of about 60 ortets of each full-sib family that had shown a high rooting ability in previous experiments. In May 1999, rooted cuttings from the 60 ortets per family were moved outdoors and grown for about 12 months in 164-ml containers (Ray-Leach Super-Cells, Steuwe and Sons, Corvallis, OR) before transplanting two ramets per ortet into 12-l containers in May 2000. Hedges were pruned to a height of 15 cm in July 2000. Nutrient treatments were as described previously.

For all experiments, terminal stem cuttings were collected from the hedged ramets of both full-sib families before 1200 h, wrapped in moist paper towels and stored in insulated coolers. Coolers were placed in a cold room maintained at 4° C for Experiments 1 and 3 (winter). For Experiments 2 and 4 (spring), coolers were placed under the propagation bench overnight at ambient greenhouse temperatures. Before setting them in the rooting medium to a depth of 1 cm, cuttings were recut from the proximal portions to a final length of 9 cm, and the basal 1 cm was dipped for 3 s in either 10 mM 1-naphthaleneacetic acid (NAA; 1.86 g l^{-1} in 30% ethanol v/v) for Experiments 1 and 3, or 2.5 mM NAA (0.46 g l^{-1} in 20% ethanol v/v) for Experiments 2 and 4. Needles were not removed from the basal portions of the cuttings that were inserted into the rooting medium.

Propagation environment

Experiments were conducted in a clear polyethylene-covered greenhouse in natural light. For Experiments 2 and 4, irradiance was decreased 60% by placing shade cloth over the greenhouse. Heating and cooling systems were set to maintain the day/night air temperature at 23–26/20–23 °C. Cuttings were misted intermittently at a variable frequency related inversely to the relative humidity (RH) in the greenhouse. Variable frequencies were defined by designating minimum (60% RH) and maximum (99% RH) off times between misting applications. Off times for intermediate humidity values were calculated based on a linear function. The minimum and maximum off times varied according to the time of day. For the period from 0600 to 0900 h, the minimum and maximum off times were 10 and 35 min, respectively. For the periods from 0900 to 1800 h, 1800 to 2100 h and 2100 to 0600 h, minimum and maximum off times were 8 and 24 min, 10 and 40 min, and 60 and 240 min, respectively. An environmental management software package (Q-Com, Irvine, CA) calculated mist frequency and triggered a traveling gantry (boom) system (ITS, McConkey, Mt. Puyallup, WA) to apply mist. Misting frequency (number of boom passes) was similar for all cuttings within each experiment; however, boom traveling speeds were altered to create different mist application treatments. For each boom speed, mist application (ml m^{-2} per boom pass) was calculated by dividing the total output for all nozzles (258 ml min^{-1} per nozzle × 26 nozzles) (TeeJet nozzle #800067, Spraying Systems, Neuvo, CA) by the area covered by the boom in 1 min. Main plots were surrounded by either white porous cloth (Experiments 1 and 2) or clear polyethylene plastic (Experiments 3 and 4) to minimize environmental gradients within the greenhouse and to separate treatments. Individual plots were surrounded by two rows of border cuttings.

Effects of mist treatment and Ψ_{sub} on Ψ_{cut} and rooting percentage (Year 1)

Experiments 1 and 2 investigated the effects of two mist treatments and four Ψ_{sub} treatments on Ψ_{cut} and rooting of stem cuttings. The design for each experiment was a split-plot with two mist treatments as the main plots and two replications of each mist treatment. Cuttings in the high-mist treatment (HM) received about 40% more mist than cuttings in the low-mist treatment (LM) during each boom pass. Four Ψ_{sub} treatments: wet, intermediate, dry and a control were the subplots. Each Ψ_{sub} treatment in a replication of mist was represented as two separate rooting tubs (subsamples within subplots). The Ψ_{sub} treatments were created by using rooting tubs of various heights for each treatment (wet = 15.2 cm, intermediate = 30.5 cm and dry = 43.2 cm) filled with fine silica sand (BX-30, Foster Dixiana, Columbia, SC). After watering each rooting tub to container capacity, the different distances between the perched water tables at the bases of the containers and the rooting zones at the tops of the sand columns in each container created the three Ψ_{sub} treatments. To maintain Ψ_{sub} at the desired treatment threshold values, tensiometers (Irrometer, Santa Monica, CA) monitored Ψ_{sub} and then triggered supplemental irrigation delivered through drip irrigation emitters (360° shrub-bubblers, Antelco, Sydney, Australia) placed at the sand surface in the rooting containers. One tensiometer was placed in each of the wet, intermediate and dry Ψ_{sub} treatments (six tensiometers total) in one replication of each mist treatment. Tensiometers were inserted so that the top of the porous ceramic tip was at the surface of the sand and the base was 3.8 cm below the surface. This allowed measurement of Ψ_{sub} near the basal portion of the stem cuttings. When Ψ_{sub} for each treatment decreased below the set critical values (described in next section), supplemental irrigation was applied to both replications for that Ψ_{sub} treatment within a mist treatment. To serve as a control, a commonly used medium, peat:perlite (2:3 v/v), was placed in a container with a height equal to the intermediate Ψ_{sub} treatment with no supplemental irrigation provided.

Twenty-one hundred cuttings, bulked from the two families, were collected for each experiment and about 120 stem cuttings were placed in each subplot (60 cuttings per subsample × 2 subsamples × 4 Ψ_{sub} treatments × 2 mist treatments × 2 replications = 1920 total stem cuttings set). We used a pressure chamber (Scholander et al. 1965) (SoilMoisture Equipment, Santa Barbara, CA) to measure Ψ_{cut} destructively at 0500 and 1400 h on one cutting per subsample 7, 14, 21, 28 and 35 days after setting (DAS). Cuttings selected randomly for Ψ_{cut} measurements were replaced to maintain canopy dynamics. We also measured Ψ_{sub} in each subsample at these times. The means of Ψ_{cut} and Ψ_{sub} for the two subsamples at two measurement times each day for the 35-day measurement period provided subplot water potential values. The percentage of cuttings that had not been sampled and produced at least one root ≥ 1 mm was recorded for each subplot 70 DAS.

Experiment 1 Juvenile hardwood stem cuttings were collected on January 5, 2001 and stored until set on January 11, 2002. Chronologically, stem cutting material was 4.5 years old from seed, although cuttings were collected from hedges that had been propagated vegetatively 18 months previously. Cuttings in the HM treatment received 121 ml m^{-2} of mist, whereas cuttings in LM received 72 ml m^{-2}, about 40% less during each boom pass. Mist treatments remained constant for 36 DAS, then HM was reduced to 102 ml m^{-2} until 50 DAS and finally reduced to 72 ml m^{-2} for the remainder of the experiment. The LM treatment remained unchanged during the experiment.

The Ψ_{sub} treatments were calibrated to –1.6 kPa (wet), –2.8 kPa (intermediate) and –3.8 kPa (dry) corresponding to sand column heights in the rooting containers of 10.2, 25.4 and 38.1 cm, respectively. The Ψ_{sub} treatments were relatively stable over the 35-day measurement period (Figure 1).

Experiment 2 Softwood stem cuttings were collected on June 22, 2001, stored overnight, and set the following day. Stem cutting material was 5 years from seed, although cuttings were collected from hedges that had been propagated vegetatively 2 years previously. Because HM stem cuttings in Experiment 1 remained continuously wet, modifications were made to the mist and Ψ_{sub} treatments in Experiment 2. During the first 35 DAS in the HM treatment, boom speed was varied manually to apply 68–121 ml m^{-2} of mist per boom pass so that the fo-

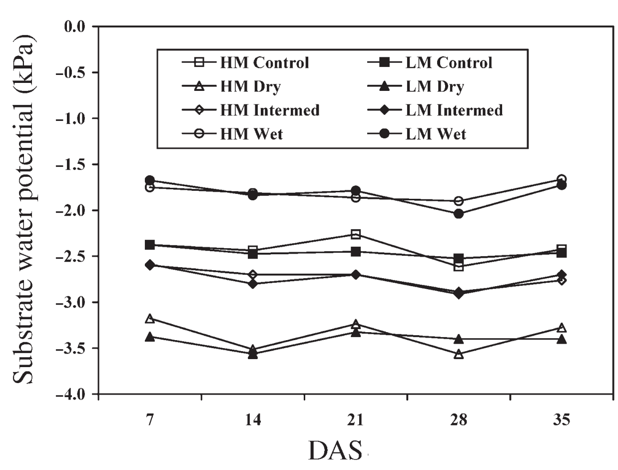

Figure 1. Mean substrate water potential (for measurements taken at 0500 and 1400 h) 7, 14, 21, 28, or 35 days after setting (DAS) for juvenile hardwood stem cuttings of loblolly pine rooted under two mist treatments (high mist (HM) and low mist (LM)) and four substrate water potential treatments (control, dry, intermediate or wet) in January 2001 (Experiment 1).

liage of the cuttings appeared dry just before the next boom pass. Simultaneously, mist application in LM was adjusted to apply 40% less mist than HM. Thiry-six DAS, mist applications in both HM and LM were adjusted to 45 ml m^{-2} for the remainder of the experiment. The Ψ_{sub} treatments were calibrated to –1.8 kPa (wet), –2.5 kPa (intermediate) and –3.5 kPa (dry) by altering the heights of the sand columns, and irrigated to maintain Ψ_{sub} within the desired range as described above. The Ψ_{sub} treatments remained constant during the measurement period, except for the control treatment where Ψ_{sub} fluctuated depending on mist treatment. Because Ψ_{sub} in the control treatment was not maintained by supplemental irrigation, it averaged –2.6 kPa in HM, and –3.1 kPa in LM. The remaining Ψ_{sub} treatments were significantly different from one another, yet similar between mist treatments for each Ψ_{sub} treatment as in Experiment 1 (data not shown).

Effect of mist treatment on Ψ_{cut} *and rooting percentage (Year 2)*

Experiments 3 and 4 tested the effects of mist treatment on Ψ_{cut} and rooting percentage. Each experiment was a randomized complete block design with two replications of six mist treatments. The mist treatments were 45, 61, 75, 102, 147 and 310 ml m^{-2} of mist application per boom pass and remained constant for the duration of the experiment. The value of Ψ_{sub} was set at –2.2 kPa for all mist treatments and maintained as described for Experiments 1 and 2. A Ψ_{sub} of –2.2 kPa was created by placing 12.5 cm of fine silica sand in 91 (length) × 61 (width) × 20 cm (height) black plastic super tubs (Rosti OS, Irving, TX).

About 100 stem cuttings were set in each plot. A pressure chamber was used to measure Ψ_{cut} destructively every 3 h between 0500 and 2300 h (seven measurements) on two cuttings per plot 7, 14, 21, 28 and 35 DAS (the 35-DAS measurements were made only in Experiment 4). Data for Ψ_{cut} were averaged over the seven measurement times each day and then over the 28- (Experiment 3) or 35-day (Experiment 4) measurement period. We measured Ψ_{sub} at 0500 and 1400 h on the same DAS and values remained at –2.4 ± 0.2 kPa among plots during these periods. The percentage of cuttings that had not been sampled and produced at least one root ≥ 1 mm was recorded for each plot 70 DAS.

Experiment 3 Hardwood stem cuttings were collected on February 15, 2002, stored and then set on April 5, 2002 (1200 total stem cuttings). Stem cutting material was 5.5 years old from seed, although stem cuttings were collected from hedges that had been propagated vegetatively 2.5 years previously.

Experiment 4 Three thousand softwood stem cuttings were collected on June 25, 2002 and set the following day. Stem cutting material was 6 years old from seed, although cuttings were collected from hedges that had been propagated vegetatively 3 years previously. One hundred stem cuttings were placed in each of two rooting tubs (subsamples) per plot for a total of 2400 cuttings. There were two rooting tubs per replication to provide enough cuttings for simultaneous measurements of photosynthesis and gas exchange (A.V. Lebude, unpublished data). Plot means were calculated from the means for Ψ_{cut}, Ψ_{sub} and rooting percentage for subsamples (2 rooting tubs).

Statistical analyses

Data were tested for normality and homogeneity of variances by univariate procedures (Steel et al. 1997) in SAS (SAS Institute, Cary, NC). In all experiments, data for Ψ_{cut} were not distributed normally, but had equal variances among treatments. Despite numerous transformations, normality was not improved significantly. However, the transformation that improved distribution according to visual examination of plots was used in a parallel analysis with the untransformed data. No differences were found between the two analyses for any experiment. Data for rooting percentage were distributed normally and had homogeneous variances among treatments; however, the arcsine square-root-transformed values were used in a parallel analysis. The outcomes of both analyses were similar. Therefore, all test statistics and means presented are based on the untransformed data of Ψ_{cut} and rooting percentage.

For Experiments 1 and 2, the main effects and interactions of mist and Ψ_{sub} on Ψ_{cut} and percent rooting were determined by analysis of variance. The relationship between Ψ_{cut} and Ψ_{sub} was determined by regression analysis. For Experiments 3 and 4, the relationship between Ψ_{cut} and mist application, and the relationship between percent rooting and mist application were determined by regression analysis. For all experiments, we examined the relationship of Ψ_{cut} as the independent variable with rooting percentage.

Results

Effects of mist treatment and Ψ_{sub} *on* Ψ_{cut} *and rooting percentage (Experiments 1 and 2)*

In Experiments 1 and 2, Ψ_{cut} was significantly affected by both mist and Ψ_{sub} treatments ($P = 0.10$) (Table 1). Values of Ψ_{cut} increased (became less negative) across all Ψ_{sub} treatments when mist application increased 40% from LM to HM (Figure 2). In Experiment 1, but not Experiment 2, Ψ_{cut} was significantly affected by the mist × Ψ_{sub} treatment interaction (Table 1). Therefore, the effect of Ψ_{sub} on Ψ_{cut} is shown by mist treatment for Experiment 1, whereas the main effect of Ψ_{sub} is shown for Experiment 2 (Figure 2). As Ψ_{sub} increased, the rate of increase in Ψ_{cut} was similar for cuttings in the combined mist treatments in Experiment 2 and for LM cuttings in Experiment 1, as indicated by similar regression slopes ($b = 0.20$ for Experiment 2 and $b = 0.21$ for LM in Experiment 1) (Figure 2). For HM cuttings in Experiment 1, however, the rate of increase in Ψ_{cut} with increasing Ψ_{sub} was significantly less ($b = 0.09$).

Mean rooting was 23 and 48% for Experiments 1 and 2, respectively. In Experiment 1, Ψ_{sub} and the mist × Ψ_{sub} interaction significantly affected rooting percentage (Table 1). In HM, mean rooting was 5 and 32% for cuttings in the wet and dry treatments, respectively. In contrast, mean rooting in LM was 42 and 22% for cuttings in the wet and dry treatments, re-

Table 1. Values of *P* for the analysis of variance for cutting water potential (Ψ_{cut}) and percent rooting of juvenile hardwood (Experiment 1) and softwood (Experiment 2) stem cuttings of loblolly pine rooted in two mist treatments and four substrate water potential (Ψ_{sub}) treatments. Bold values are statistically significant ($P < 0.1$). Error A was used as the error term to test the main effect of replication and mist treatment, and Error B was used as the error term to test the main effect of Ψ_{sub} and the mist × Ψ_{sub} interaction.

Source	df	Experiment 1		Experiment 2	
		Ψ_{cut}	Rooting (%)	Ψ_{cut}	Rooting (%)
Replication	1	0.53	0.28	0.46	0.12
Mist treatment	1	**0.08**	0.17	**0.02**	**0.02**
Error A	1	0.14	0.32	0.68	0.85
Ψ_{sub}	3	**0.01**	**0.04**	**0.01**	0.40
Mist treatment × Ψ_{sub}	3	**0.09**	**0.01**	0.50	0.88
Error B	6				

spectively. In Experiment 2, mist treatment, but not Ψ_{sub}, significantly affected rooting percentage (Table 1). In this experiment, rooting percentage was higher for HM cuttings (60%) than for LM cuttings (35%), regardless of Ψ_{sub} treatment.

In Experiment 1, rooting percentage was related moderately with both the linear and quadratic terms of Ψ_{cut} ($P = 0.01$, $r^2 = 0.56$, for the equation containing both terms) (Figure 3A). Rooting percentage increased to about 35% as Ψ_{cut} increased from –0.8 to –0.5 MPa. However, percent rooting then decreased as Ψ_{cut} continued to increase to –0.2 MPa (Figure 3A). For cuttings in Experiment 2, rooting increased linearly as Ψ_{cut} increased (Figure 3B). Although overall rooting was higher for Experiment 2 than for Experiment 1, the highest rooting percentages were found in cuttings of several treatment combinations, with mean Ψ_{cut} between –0.4 and –0.6 MPa, similar to Experiment 1.

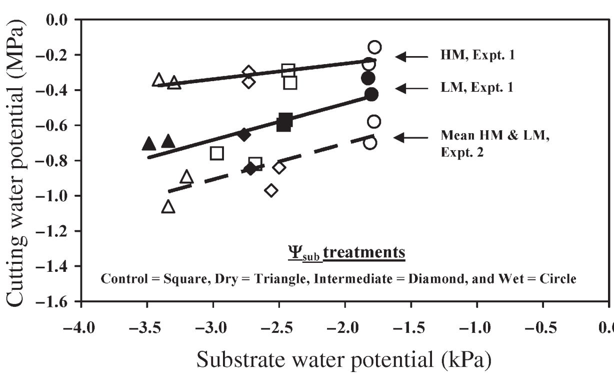

Figure 2. Effect of mist treatment and substrate water potential (Ψ_{sub}) on mean cutting water potential (Ψ_{cut}) of juvenile hardwood (Experiment 1, conducted in January 2001) and softwood (Experiment 2, conducted in June 2001) stem cuttings of loblolly pine. Solid regression lines represent Experiment 1. The open symbols in Experiment 1 represent the high mist (HM) and solid symbols the low mist (LM) treatments. The dashed regression line represents Experiment 2, and the open symbols represent the means of HM and LM. Symbols are means ($n = 20$ for Experiment 1, and $n = 40$ for Experiment 2) of Ψ_{cut} and Ψ_{sub} measured at 0500 and 1400 h 7, 14, 21, 28 and 35 days after setting in each plot. Regression equations are Ψ_{cut} (HM) = –0.07 + $0.09\Psi_{sub}$ ($P = 0.03$, $r^2 = 0.58$) and Ψ_{cut} (LM) = –0.07 + $0.21\Psi_{sub}$, $P = 0.02$, $r^2 = 0.61$, for Experiment 1 and Ψ_{cut} (combined HM and LM) = –0.30 + $0.20\Psi_{sub}$, $P = 0.04$, $r^2 = 0.61$, for Experiment 2.

Effects of mist treatment on Ψ_{cut} *and rooting percentage (Experiments 3 and 4)*

Over the 28- and 35-day measurement periods in Experiments 3 and 4, respectively, Ψ_{cut} tended to follow a similar diurnal pattern for each mist treatment (Figure 4): it decreased between 0800 and 1700 h and increased between 1700 and

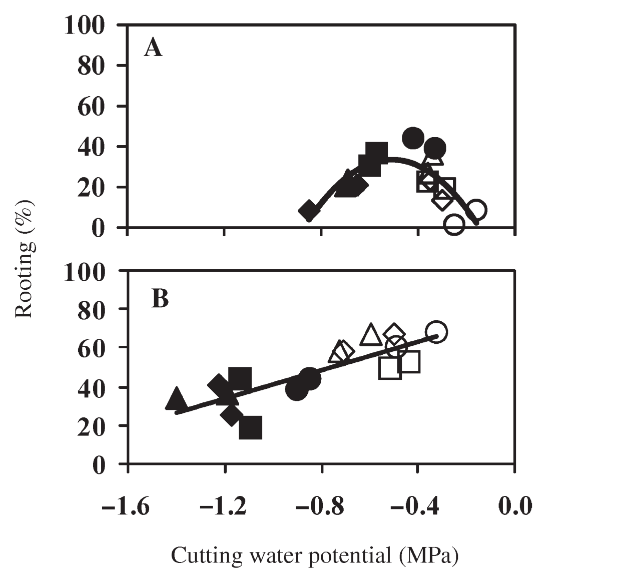

Figure 3. Relationship between rooting percentage and cutting water potential (Ψ_{cut}) of juvenile (A) hardwood (Experiment 1, conducted in January 2001) and (B) softwood (Experiment 2, conducted in June 2001) stem cuttings of loblolly pine rooted under high mist (HM = open symbols) and low mist (LM = solid symbols) and four substrate water potential treatments (control = ■, □; dry = ▲, △; intermediate = ◆, ◇; and wet = ●, ○). Symbols are means ($n = 20$) of Ψ_{cut} measured at 0500 and 1400 h 7, 14, 21, 28, or 35 days after setting in each plot. Values for the quadratic term, Ψ_{cut}^2, in Experiment 1 were generated for Ψ_{cut} in each plot initially, and then averaged similarly to the linear term. The regression equations are (A) Rooting (%) = –32.80 – $261.66\Psi_{cut} - 257.47\Psi_{cut}^2$, $P = 0.01$, $R^2 = 0.56$ and (B) Rooting (%) = 77.49 + 36.2 Ψ_{cut}, $P = 0.01$, $r^2 = 0.67$.

0800 h. Although variation occurred in both experiments, cuttings receiving 310 ml m^{-2} of mist had minimal change in Ψ_{cut} either during the day or over the measurement period (Figure 4). In both experiments, mean Ψ_{cut} (across all times and measurement dates) was strongly related to the log of mist application (Experiment 3, R^2 = 0.97 and Experiment 4, r^2 = 0.96) (Figure 5). The sharpest increase in Ψ_{cut} was between 45 and 102 ml m^{-2} of mist per application, whereas above 147 ml m^{-2}, the increase was more gradual.

Mean rooting was 73 and 63% for Experiments 3 and 4, respectively. Rooting percentage was significantly affected by both the linear and quadratic terms of mist treatment in Experiment 3 (P = 0.03, R^2 = 0.54, for the equation containing both terms), but neither the linear nor the quadratic terms affected rooting percentage in Experiment 4 (Figure 6). In Experiment 3, rooting increased to 94% in a mist treatment of 147 ml m^{-2} per boom pass, and then declined as mist application increased (Figure 6).

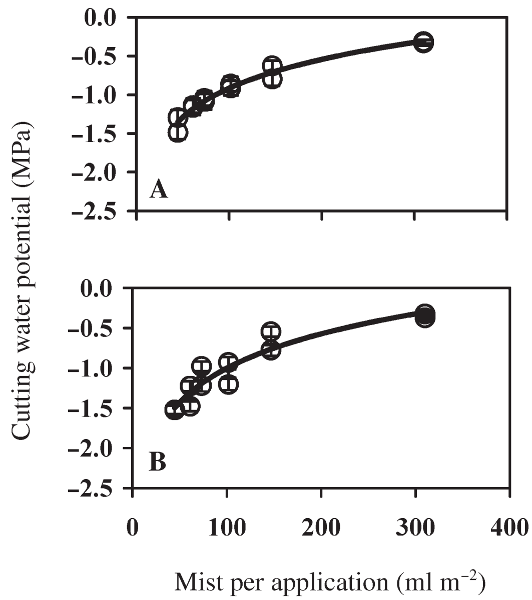

Figure 5. Relationship between cutting water potential (Ψ_{cut}) and 45, 61, 73, 102, 147, or 310 ml m^{-2} of mist per application for juvenile (A) hardwood stem cuttings in April 2002 (Experiment 3) and (B) softwood stem cuttings of loblolly pine in June 2002 (Experiment 4). Symbols are mean (n = 56 for Experiment 3, and n = 70 for Experiment 4) Ψ_{cut} for each replication recorded seven times per day, 7, 14, 21, 28, or 35 (day 35 measured for Experiment 4 only) days after setting cuttings in each plot. The regression equations are (A) Ψ_{cut} = –3.39 + 0.54ln(ml m^{-2}), P = 0.01, r^2 = 0.97, and (B) Ψ_{cut} = –3.84 + 0.62ln(ml m^{-2}), P = 0.01, r^2 = 0.96. Vertical bars = ± 1 SE (n = 4 for Experiment 3, and n = 5 for Experiment 4).

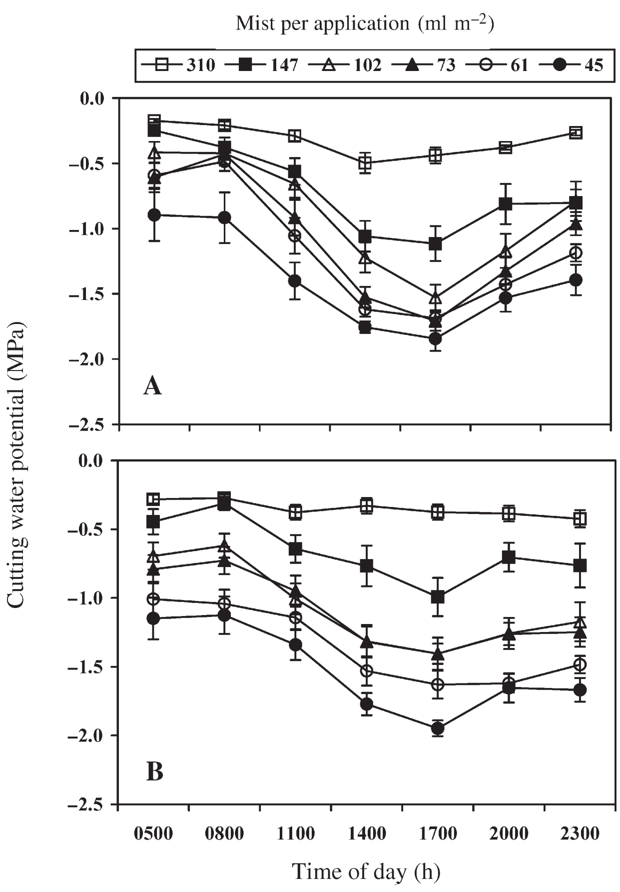

Figure 4. Cutting water potential by time of day for juvenile (A) hardwood (Experiment 3, conducted in April 2002) and (B) softwood (Experiment 4, conducted in June 2002) stem cuttings of loblolly pine receiving 45, 61, 73, 102, 147, or 310 ml m^{-2} of mist per application. Symbols are means (n = 8 for Experiment 3, and n = 10 for Experiment 4) of two replications per mist treatment averaged over 7, 14, 21, 28, or 35 (day 35 measurement for Experiment 4 only) days after setting. Vertical bars = ± 1 SE (n = 4 for Experiment 3 and n = 5 for Experiment 4).

Rooting percentage was related moderately with both the linear and quadratic terms of Ψ_{cut} in both experiments (P = 0.01, R^2 = 0.70, for equations containing both terms in both experiments) (Figure 7). In Experiment 3, the highest rooting percentage (90%) occurred for cuttings with a mean Ψ_{cut} between –0.5 and –1.0 MPa. In Experiment 4, the highest rooting

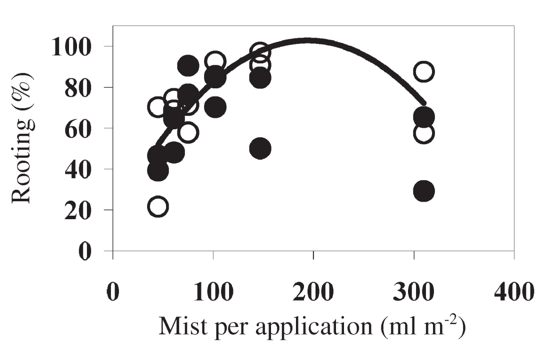

Figure 6. Effect of 45, 61, 73, 102, 147, or 310 ml m^{-2} of mist per application on percent rooting of juvenile hardwood (Experiment 3, conducted in April 2002, ○) and softwood (Experiment 4, conducted in June 2002, ●) stem cuttings of loblolly pine. The regression equation is Rooting (%) Experiment 3 = 16.36 + 0.89(ml m^{-2}) – 0.0023 (ml m^{-2})^2, P = 0.03, R^2 = 0.54. Mist application did not significantly affect percent rooting during Experiment 4.

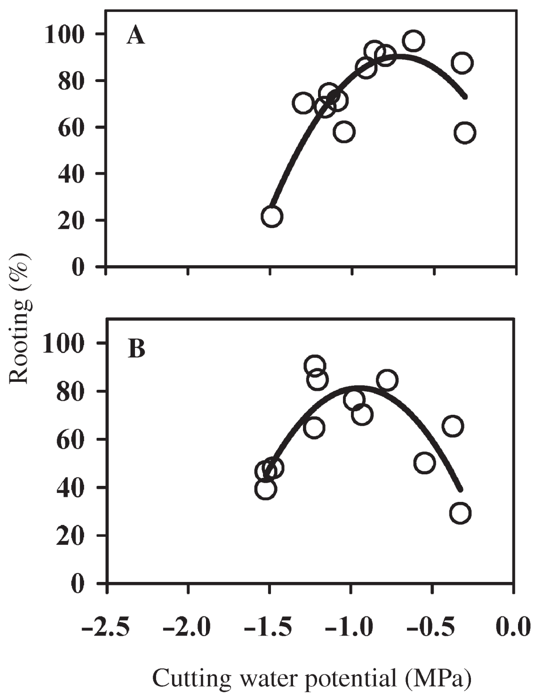

Figure 7. Relationship between rooting percentage and mean cutting water potential (Ψ_{cut}) of juvenile (A) hardwood (Experiment 3, April 2002) and (B) softwood (Experiment 4, June 2002) stem cuttings of loblolly pine rooted under six mist treatments. Symbols are means (n = 56 for Experiment 3, and n = 70 for Experiment 4) of Ψ_{cut} recorded seven times per day 7, 14, 21, 28, or 35 days after setting cuttings (Day 35 measurement for Experiment 4 only) in each plot. Values for the quadratic term, Ψ_{cut}^2, were generated for Ψ_{cut} in each plot initially, and then averaged similarly to the linear term. The regression equation for (A) is Rooting (%) = 9.26 – 261.94Ψ_{cut} – 152.44 Ψ_{cut}^2, P = 0.01, R^2 = 0.70, and for (B) Rooting (%) = –31.49 – 283.57Ψ_{cut} – 140.03Ψ_{cut}^2, P = 0.01, R^2 = 0.70.

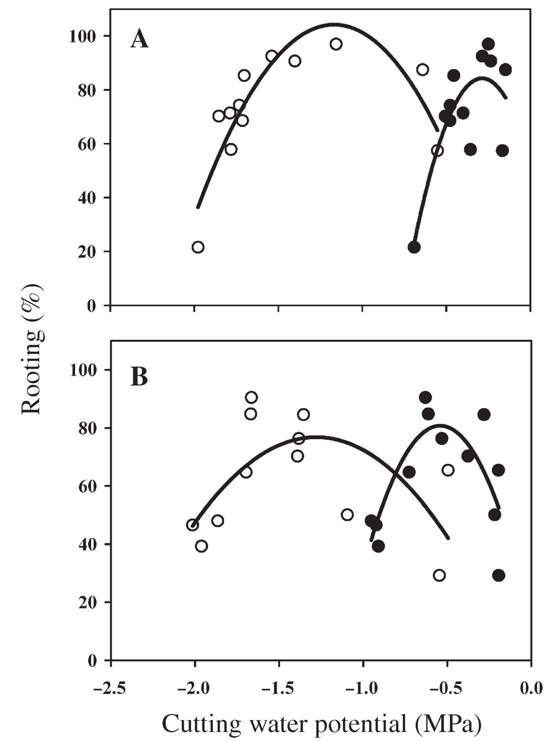

Figure 8. Relationship between rooting percentage and mean minimum (open symbols) and maximum (solid symbols) cutting water potential (Ψ_{cut}) for juvenile (A) hardwood (Experiment 3 conducted in April 2002) and (B) softwood (Experiment 4 conducted in June 2002) stem cuttings of loblolly pine. Symbols are means of the minimum and maximum daily Ψ_{cut} values recorded 7, 14, 21, 28, or 35 days (Day 35 for Experiment 4 only) after setting cuttings in each plot (n = 4 for Experiment 3 and n = 5 for Experiment 4). Values for the quadratic term, Ψ_{cut}^2, were generated for Ψ_{cut} in each plot initially, and then averaged similarly to the linear term. The regression equations for (A) are Rooting (%) = –31.24 – 237.57(Minimum Ψ_{cut}) – 102.34 (Minimum Ψ_{cut})^2, P = 0.01, R^2 = 0.78, Rooting (%) = 63.95 – 138.01(Maximum Ψ_{cut}) – 214.04 (Maximum Ψ_{cut})^2, P = 0.02, R^2 = 0.61, and for (B) Rooting (%) = –17.42 – 154.57(Minimum Ψ_{cut}) – 60.89(Minimum Ψ_{cut})^2, P = 0.07, R^2 = 0.45, and Rooting (%) = 14.04 – 241.21(Maximum Ψ_{cut}) – 196.94(Maximum Ψ_{cut})^2, P = 0.01, R^2 = 0.61.

percentage (80%) occurred for cuttings with a mean Ψ_{cut} between –0.7 and –1.2 MPa.

Minimum (most negative) and maximum daily Ψ_{cut} were selected from the weekly measurements for each plot, regardless of the time of day, and averaged over the 28- (n = 4) or 35-day (n = 5) measurement period. In Experiment 3, rooting percentage was moderately to highly correlated with minimum Ψ_{cut} (R^2 = 0.78, P = 0.01) (Figure 8A). The regression equation predicted that 80% or more cuttings rooted when mean daily minimum Ψ_{cut} was between –0.7 and –1.65 MPa (Figure 8A). In Experiment 4, rooting percentage was related closely to minimum Ψ_{cut} (R^2 = 0.45, P = 0.07) (Figure 8B). The regression equation did not predict 80% rooting from the observed data, so 70% rooting was used as a benchmark. At 70% rooting or higher, Ψ_{cut} ranged from –0.95 to –1.60 MPa (Figure 8B), however, variation in rooting was considerable.

The relationship between rooting percentage and maximum Ψ_{cut} was moderate for both Experiments 3 (R^2 = 0.61, P = 0.02) and 4 (R^2 = 0.61, P = 0.01) (Figure 8). When cuttings in Experiment 3 experienced a mean maximum Ψ_{cut} between –0.2 and –0.4 MPa, rooting was 80%. In Experiment 4, when cuttings experienced a mean maximum Ψ_{cut} between –0.35 and –0.75 MPa, rooting was 70% or higher.

Discussion

Mist and Ψ_{sub} contributed to the Ψ_{cut} of unrooted, juvenile stem cuttings of loblolly pine. For all cuttings in Experiments 1 and 2, Ψ_{cut} increased as Ψ_{sub} increased (Figure 2). Cuttings in individual mist treatments received equal mist application, so increases in Ψ_{cut} were a result of water uptake from the substrate. Increasing mist treatment also increased Ψ_{cut} for all cuttings in all experiments (Figures 2 and 5). It has been suggested that water uptake can occur through both the aerial portion of the foliage exposed to intermittent mist application and the basal portion of the shoot inserted in the rooting substrate (Cameron and Rook 1974, Cremer and Svensson 1979, Peer and Greenwood 2001). In addition to absorption through the foliage, increased mist application could decrease transpiration, thus preventing water loss. The effect of either mist or Ψ_{sub} on Ψ_{cut} decreased for cuttings experiencing little water stress in Experiment 1, as indicated by the significant Ψ_{sub} × mist treatment interaction ($P = 0.09$).

Cuttings with the most negative Ψ_{cut} for each experiment had poor rooting percentages (Figures 3 and 7). This is consistent with reports of poor rooting of stem cuttings of many species experiencing substantial water deficit or severely decreased Ψ_{cut} (Loach 1977, Grange and Loach 1984, Rein et al. 1991, Murthy and Goldfarb 2001, Peer and Greenwood 2001). On the other hand, cuttings with the highest Ψ_{cut} in Experiments 1 (Figure 3A), 3 (Figure 7A) and 4 (Figure 7B) also rooted poorly. The one exception was in the case of cuttings in Experiment 2 in which the revised method of mist application did not produce high values of Ψ_{cut} in stem cuttings. Greenwood et al. (1980) also reported that excessive mist application decreased rooting percentage in loblolly pine; however, these authors did not report Ψ_{cut}. Our finding that juvenile stem cuttings of loblolly pine experiencing little or no water deficit root poorly has not previously been reported. In general, the deleterious effect of excessive mist on rooting may be indirect because basal portions of stem cuttings can become waterlogged and, ultimately, necrotic if the substrate is saturated. However, in our studies, this indirect effect was excluded by independently controlling mist treatment and Ψ_{sub}. Furthermore, we did not observe basal stem necrosis in any cuttings.

Our results suggest that, in juvenile stem cuttings of loblolly pine, moderate water stress imposed during rooting stimulated adventitious root formation and development. Cuttings with the highest rooting percentages had mean Ψ_{cut} values between –0.5 and –1.2 MPa (Figures 3 and 7). These results agree with rooting percentages and Ψ_{cut} values reported for other species. Juvenile stem cuttings of Alaskan yellow cedar (*Chamaecyparis nootkatensis* (D. Don) Spach.) had a mean Ψ_{cut} of –0.8 MPa (mean at 1100 h over 13 weeks prior to rooting) and a rooting percentage of 92% (Grossnickle and Russell 1993). Two treatments of stem cuttings of mountain lilac (*Ceanothus thrysiflorus* Eschsch.) had mean Ψ_{cut} values of –0.8 and –1.6 MPa (mean of 0900 and 1400 h) during the rooting period, with rooting percentages of 96 and 56%, respectively (Loach 1977). Prior to rooting, the cuttings with mean Ψ_{cut} of –0.8 MPa had a one-time minimum stress of –1.2 MPa, whereas cuttings with mean Ψ_{cut} of –1.6 MPa had a one-time minimum stress of –3.9 MPa (Loach 1977). Cuttings of both yellow cedar and mountain lilac, with the highest percentage rooting, also had mean Ψ_{cut} within the range (–0.5 to –1.2 MPa) that induced superior rooting in loblolly pine. However, it cannot be determined from all these reports whether rooting percentage was adversely affected by a one-time exposure to severe water stress, repeated severe water stress, or the mean Ψ_{cut} experienced by cuttings during the rooting period.

Stem cuttings of loblolly pine rooted ~80% to ~70%, in Experiments 3 and 4, respectively, when mean daily minimum Ψ_{cut} was more than –1.6 MPa but less than –0.90 MPa (Figure 8). Murthy and Goldfarb (2001) reported that rooting of juvenile hardwood and softwood stem cuttings of loblolly pine decreased below 80 and 70%, respectively, when cuttings experienced a one-time Ψ_{cut} less than –3.5 MPa for hardwood and less than –1.7 MPa for softwood stem cuttings. In that study, the one-time water deficit treatments were imposed in a laboratory atmosphere before cuttings were rooted under optimal conditions. Thus, water stress occurred well in advance of adventitious root formation. By contrast, treatment differences in our study occurred repeatedly and throughout the period leading to rooting. Moreover, our cuttings experienced conditions such as high irradiance and leaf temperature, which, in addition to Ψ_{cut}, may have deleterious effects in the cuttings. Therefore, juvenile stem cuttings of loblolly pine may experience moderate to severe water stress and still produce adventitious roots at high percentages, provided that the cuttings do not experience mean daily minimum Ψ_{cut} of less than –1.6 MPa.

The mechanism(s) by which Ψ_{cut} affects adventitious root formation in stem cuttings is unclear (Grange and Loach 1984, Sinclair and Ludlow 1985, Loach 1988). Cutting water potential comprises both osmotic potential and turgor pressure (Kramer and Kozlowski 1979) of the cutting, and these components may differentially affect the two general stages of adventitious root formation—root initiation and root growth and development. For example, a low Ψ_{cut} may not affect root initiation, but loss of turgor at a critical point could prevent or retard growth and development, thus decreasing the percentage of cuttings observed to be rooted. Moreover, intact seedlings of loblolly pine exposed repeatedly to water stress can undergo osmotic adjustment, which decreases the solute potential and maintains turgor at a lower Ψ (Seiler and Johnson 1985). Decreased percent rooting has been associated with lower osmotic potentials in response to high irradiance in stem cuttings of some woody species (Grange and Loach 1985). Whether osmotic adjustment occurs in stem cuttings and, if so, how it affects the two stages of adventitious root formation are unknown. Further studies measuring the effects of osmotic and turgor potentials on adventitious root initiation and development are needed to better understand these mechanisms. In addition, more investigation is necessary to extend these results to other propagation environments to test whether our finding that moderate water stress stimulates rooting percentages in loblolly pine stem cuttings has broad applicability.

In conclusion, substrate water availability and mist application affected the water status of unrooted stem cuttings of loblolly pine because cuttings absorbed water from the substrate and either absorbed water through the foliage above the substrate, or experienced reduced transpirational demand as a result of misting. Substrate water content affected rooting percentage to some extent when mist was suboptimal or applied excessively. However, when adequate soil water was available, mist treatment was the overriding factor determining rooting percentage. When Ψ_{sub} was held constant across mist treatments, mist treatment determined the Ψ_{cut} of unrooted stem cuttings and Ψ_{cut}, in turn, strongly affected rooting percentage. Stem cuttings of loblolly pine rooted at high percentages when experiencing moderate water stress before initiating adventitious roots. Cuttings experiencing more severe water stress, or none, rooted poorly. We conclude that the rooting environment should impose a moderate water stress on loblolly pine stem cuttings if optimum rooting is to be achieved.

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